A-1

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 15/00, 7/08, A61K 37/02	A1	(11) International Publication Number: WO 94/06828 (43) International Publication Date: 31 March 1994 (31.03.94)

(21) International Application Number: PCT/US93/08456

(22) International Filing Date: 9 September 1993 (09.09.93)

(30) Priority data:
07/943,799 11 September 1992 (11.09.92) US

(71) Applicant: WASHINGTON UNIVERSITY SCHOOL OF MEDICINE [US/US]; Washington University Medical Center, Att. Paul Allen, 660 South Euclid Avenue, St. Louis, MO 63110 (US).

(72) Inventors: ALLEN, Paul, M. ; 704 Fieldstone Terrace, Webster Groves, MO 63119 (US). SLOAN-LANCASTER, Joanne ; 7430 Grant Village Dirve, St. Louis, MO 63123 (US). EVAVOLD, Brian, D. ; 520 Shepards Crossing, Ballwin, MO 63021 (US).

(74) Agents: CHANNING, Stacey, L. et al.; Immulogic Pharmaceutical Corporation, 610 Lincoln Street, Waltham, MA 02154 (US).

(81) Designated States: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PEPTIDES TOLERIZING T-CELLS AND COMPOSITIONS THEREOF

(57) Abstract

The present invention provides a tolerizing peptide and a therapeutic composition containing at least one such tolerizing peptide which when administered to an individual in a therapeutically effective amount is capable of tolerizing at least a portion and preferably all of the T cells of said individual which are specific for an antigen (e.g. an allergen, an autoantigen or a transplantation antigen), said tolerizing peptide having the ability to combine with MHC proteins on antigen presenting cells (APC) and cause binding of T cell receptors on at least a portion of the T cells of an individual to said peptide-MHC complex. Said tolerizing peptide preferably has the ability to cause no detectable or minimal proliferation of at least a portion of the T cells of an individual which are specific for said antigen when said peptide is administered in vivo in immunogenic form. The invention also provides a method for treating sensitivity to a particular antigen in an individual and a method for tolerizing at least a portion of a population of T cells in an individual which are specific for an antigen.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

PEPTIDES TOLERIZING T-CELLS AND COMPOSITIONS THEREOF

BACKGROUND OF THE INVENTION

Several apparently disparate pathological states, such as allergy, transplant rejection, and autoimmune diseases share the common characteristic of being caused by deleterious immune reactions, which at least in part involve activation of T cells. The cascade of molecular events leading to this T cell activation is initiated by the formation of complexes between antigenic peptides (a processed peptide derived from disease-causing antigenic protein comprising at least one T cell epitope) and major histocompatibility complex molecules (MHC). These antigen-MHC complexes are ligands that, in turn, are recognized by T cell receptor (TCR) on the surface of T cells which are specific for the disease-causing antigen. Engagement of the TCR in the trimolecular antigen-MHC-TCR complex together with a costimulatory signal results in activation of the T cell which is followed by a series of molecular events characteristic of T cell activation, such as increase in tyrosine phosphorylation, Ca 2+ influx, inositol phosphate turnover, the synthesis of cytokines (i.e. IL-2, IL-3, gamma interferon) and cytokine receptors, and T cell proliferation.

Several investigators have sought to develop treatments which interfere with this recognition process by using high affinity MHC-binding peptides that cause inhibition of T cell activation by blocking the antigen-binding site of MHC molecules. Other investigators have attempted to devise treatments which result in tolerization of T cells by various mechanisms which include engaging the TCR with an antigenic peptide derived from the

target antigen which is bound to the appropriate MHC complex but without the appropriate costimulatory signal, and which therefore causes the target T cells to become tolerized. Tolerized T cells will not proliferate when stimulated with low antigen concentrations and will not produce IL-2 under any conditions. It would be advantageous to provide treatment for allergy, transplantation rejections or autoimmune disease which causes tolerization of the targeted antigen T cells (i.e. the T cells do not proliferate or proliferate only minimally and further do not perform any of the other functions associated with T cell activation discussed above) and moreover, it would be advantageous if T cell tolerization can occur in the presence or absence of the appropriate costimulatory signal.

SUMMARY OF THE INVENTION

The present invention provides a tolerizing peptide and a therapeutic composition containing at least one such tolerizing peptide which when administered to an individual in a therapeutically effective amount is capable of tolerizing at least a portion of the T cells of said individual which are specific for an antigen (e.g. an allergen, an autoantigen or a transplantation antigen), said tolerizing peptide having the ability to combine with MHC on antigen presenting cells (APC) and cause binding of T cell receptors on at least a portion of the T cells of an individual to said peptide-MHC complex and having at least one and preferably all of the following properties: 1) the ability to cause no detectable or minimal proliferation of at least a portion of the T cells of an individual which are specific for said antigen in an in vitro assay utilizing said peptide and histocompatible antigen-presenting cells; 2) the ability to cause no detectable or minimal production of IL-2 in an in vitro

assay utilizing a portion of the T cells of an individual which are specific for said antigen, said peptide, and histocompatible antigen-presenting cells; 3) the ability to cause no detectable or minimal production of IL-3 in an in vitro assay utilizing a portion of the T cells of an individual which are specific for said antigen, said peptide, and histocompatible antigen presenting cells: and 4) the ability to cause no detectable or minimal production of gamma IFN in an in vitro assay utilizing a portion of the T cells of an individual which are specific for said antigen, said peptide, and histocompatible antigen presenting cells. Said tolerizing peptide preferably has the ability to cause no detectable or minimal proliferation of a least a portion of the T cells of an individual which are specific for said antigen when said peptide is administered in vivo in immunogenic form. A therapeutic composition of the present invention preferably comprises a sufficient number of tolerizing peptides to render substantially all of the T cells of such individual which are specific for said antigen unresponsive to said antigen.

The invention also provides a method for treating sensitivity to a particular antigen in an individual by administering to the individual, preferably in nonimmunogenic form, at least one therapeutic composition of the present invention in an amount effective to render at least a portion of, and preferably all of the T cells of the individual which are specific for said antigen unresponsive to said antigen. At least two different therapeutic compositions of the invention can be administered simultaneously or sequentially to the individual. Another embodiment of the present invention provides a method for inhibiting at least a portion of an antigen specific antibody response by the immune system of an individual by administering to the individual, preferably in nonimmunogenic form, at least one therapeutic

composition of the present invention in an amount effect to render at least a portion of the T cells of the individual which are specific for said antigen unresponsive to said antigen.

In yet another embodiment of the invention, there is provided a method of designing a tolerizing peptide of the invention and a method for tolerizing at least a portion of a population of T cells in an individual which are specific for an antigen utilizing said tolerizing peptide.

DESCRIPTION OF THE DRAWINGS

Fig 1a. is a graphic representation of a T cell proliferation assay of T cell clone PL-17 to a wild type peptide of the murine hemoglobin Bd minor chain (HbB(64-76) designated in the graph as wt Hb) or the substituted HbB(64-76) peptide, Ser70, of the invention and using I-E ^{k}-transfected L cell fibroblasts as antigen presenting cells (APC).

Fig 1b is similar to Fig 1a except that two substituted HbB(64-76) peptides, Ser70 and Gln72, are used in the T cell proliferation assay.

Fig 2a is a graphic representation of a bioassay for IL-2 production by Th 1 clone PL-17 stimulated with Wt Hb or substituted peptide, Ser70 and quantitated as proliferation of the IL-2 dependent cell line CTLL; and CH27 B cell lymphoma were used as APC.

Fig 2b is similar to Fig. 2a except that two substituted HbB(64-76) peptides, Ser70 and Gln72 are used in the assay.

Fig. 3a is a graphic representation in bar graph form of a bioassay for gamma IFN production by Th 1 clone PL17 stimulated with Wt Hb and substituted peptide, Ser70; L cell fibroblasts were used as antigen presenting cells and gamma IFN was measured using an ELISA .

Fig 3b is a graphic representation in line graph form of a bioassay for gamma IFN production similar to that described in Fig. 3a except that substituted peptide Gln72 was also tested.

Fig 4 is a graphic representation of a bioassay for IL-3 production by Th 1 clone PL17 stimulated with Wt Hb or substituted peptide, Ser70; IL-3 production was quantitated as proliferation of the IL-3 dependent cell line, GG1.12, and B10.BR/sgSnj spleen cells were used as APC.

Fig. 5a is a graphic representation of the results of a tolerance assay using Th 1 clone PL-17 which had been incubated overnight with either no antigen (No Ag o/n), 50 uM Ser 70, or 50 uM Gln72, and rested 1 day before being challenged with wild type Hb peptide(64-76), DCEK-Hi7 L cells or B10.BR/SgSnj spleen cells were used as APC.

Fig 5b is a graphic representation of the results of a tolerance assay using Th 1 clone PL-17 which had been incubated overnight with either no antigen (No Ag o/n), or 50 uM Ser 70 and rested 3 days before being challenged with wild type Hb peptide(64-76); DCEK-Hi7 L cells or B10.BR/SgSnj spleen cells were used as APC.

Fig. 5c is a graphic representation of the results of a tolerance assay using Th 1 clone PL-17 which had been incubated overnight with either no antigen (No Ag o/n), or 50 uM Ser 70 and rested 5 days before being challenged with wild type Hb peptide(64-76); DCEK-Hi7 L cells or B10.BR/SgSnj spleen cells were used as APC.

Fig. 5d is a graphic representation of the results of a tolerance assay using Th 1 clone PL-17 which had been incubated overnight with either no antigen (No Ag o/n), or 50 uM Ser 70 and rested 7 days before being

challenged in a proliferation assay with Hb peptide(64-76); DCEK-Hi7 L cells or B10.BR/SgSnj spleen cells were used as APC.

Fig. 6 is a graphic representation of the results of an assay for inositol phosphate generation by Th 1 clone PL17 cells which had been incubated with no peptide, Hb(64-76), Ser70 or Gln72 and in which either DCEK-Hi7 L cells or B10.BR/SgSnj spleen cells were used as APC; total free inositol phosphate was quantitated by scintillation counting.

Fig. 7a is a graphic representation of the results of a tolerance assay using Th 1 clone Pl-17 cells which had been incubated over night with either no antigen(No Ag o/n), 50 uM Ser70 (50uM s70 o/n), 50 uM Gln72 (50 uM Q71 o/n) or 100 uM Gln72 (100uM Q72 o/n) and DCEK-Hi7 L cell tranfectants as APC and subsequently challenged with Hb(64-76) as antigen.

Fig. 7b is a graphic representation of the results of a tolerance assay using Th 1 clone Pl-17 cells which had been incubated overnight with various concentrations of substituted peptide, Ser70, and DCEK-Hi7 L cell tranfectants or as APC and subsequently challenged with Hb(64-76) as antigen.

Fig. 8 is a graphic representation of the results of a tolerance assay using PL-17 cells incubated overnight with no antigen, or substituted peptide, Ser70, or with no antigen and Cyclosporin A ,or substituted peptide, Ser70, and Cyclosporin A and with DCEK-Hi7 L cells as APC and subsequently challenged with Hb(64-76) as antigen.

Fig. 9 is a graph depicting a FACScan analysis testing for the levels of IL-2 receptor on the surface of PL-17 cells which had been incubated with DCEK/Hi7 L cells alone or with substituted peptides Ser70 or Gln 72 at 50 uM.

Fig. 10 is a graph depicting a FACScan analysis testing for the levels of LFA-1 adhesion molecule on the surface of PL-17 cells which had been incubated with DCEK/Hi7 L cells alone or in conjunction with substituted peptides Ser70 or Gln 72 at a concentration of 50 uM.

Fig. 11a is a graphic representation of the results of a tolerance assay using Th 1 clone PL-17 which had been incubated overnight with either no antigen (No Ag o/n), or 50 uM Ser 70 and rested 3 days before being challenged in a proliferation assay with wild type Hb peptide(64-76).

Fig. 11b is a graphic representation of the results of a tolerance assay using Th 1 clone PL-17 which had been incubated overnight with either no antigen (No Ag o/n), or 50 uM Ser 70 and rested 3 days before being challenged in a proliferation assay with wild type Hb peptide(64-76).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a tolerizing peptide (also referred to herein as a tolerizing substituted peptide) and a therapeutic composition containing at least one such tolerizing peptide which when administered to an individual in a therapeutically effective amount is capable of tolerizing at least a portion of the T cells of said individual which are specific for an antigen (e.g. an allergen, an autoantigen or a transplantation antigen), said tolerizing peptide causing no detectable or minimal proliferation of at least a portion of the T cells of said individual which are specific for said antigen in an in vitro assay using histocompatible antigen-presenting cells. The terms "tolerization", "tolerizing", "anergy" and "anergizing" etc. are used herein means that the T cell is rendered unable to respond to stimulation with an antigen complexed to MHC. Here tolerization includes the binding of the T cell receptor to

complexes of the tolerizing peptide and the MHC. The term minimal, as used herein, when discussing minimal T cell proliferation, minimal lymphokine production, etc. refers to an amount that is not significant. Histocompatible antigen-presenting cells are antigen-presenting cells (APCs) that are histocompatible with the T cell population. This is automatic if the T cells and the APCs are obtained from the same source. However, they may also be prepared separately. For human-related assays, immortalized B-cell lines from the same subject, MHC Class II compatible cell lines, or compatible peripheral blood mononuclear cells can be used as APCs. Said tolerizing peptide preferably has the ability to cause no detectable or minimal proliferation of a least a portion of the T cells of an individual which are specific for said antigen when said peptide is administered in vivo in immunogenic form. By immunogenic form is meant a form which tends to induce an immune response, i.e., cause the activation of T cells or production of antibodies specifically immunoreactive with the peptide. It is well known that peptides administered in adjuvants tend to be immunogenic while peptides administered in the absence of adjuvant and in soluble form (nonimmunogenic form) tend to be tolerogenic. Other characteristics of tolerizing peptides of the invention include the ability to cause no detectable or minimal production of lymphokines, including but not limited to IL-2, IL-3, IL-4, gamma interferon, etc. in an in vitro assay utilizing a portion of the T cells of an individual which are specific for said antigen and histocompatible antigen-presenting cells.

A therapeutic composition of the present invention preferably comprises at least one tolerizing peptide of the invention and a pharmaceutically acceptable carrier or diluent. Pharmaceutically acceptable

diluents include saline and aqueous buffer solutions. Pharmaceutically acceptable carriers include polyethylene glycol (Wie et al., *International Archives of Allergy and Applied Immunology* 64: 84-99 (1981)) and liposomes (Strejan et al., *Journal of Neuroimmunology* 7: 27 (1984)). Such compositions will generally be administered by injection (subcutaneous, intravenous, etc.), oral administration (e.g., as in the form of a capsule), inhalation, transdermal application or rectal application. The therapeutic composition of the present invention preferably comprises a sufficient number of tolerizing peptides to render substantially all of the T cells of such individual which are specific for said antigen unresponsive to said antigen. This may turn out to be one tolerizing peptide, two tolerizing peptides, three tolerizing peptides, etc. The therapeutic compositions of the invention are administered to individuals at dosages and for lengths of time effective to reduce sensitivity of the individual to the antigen. As used herein, reduction in sensitivity can be defined as non-responsiveness or dimunition in the symptoms to the antigen (i.e. the individual would be less able to respond immunologically to the antigen as determined by clinical and/or scientific procedures). This dimunition may be subjective (i.e.,the individual feels more comfortable despite the presence of the antigen). Effective amounts of the therapeutic compositions will vary according to factors such as degree of sensitivity of the individual to the antigen, the age, sex, and weight of the individual, etc.

The invention also provides a method for treating sensitivity to a particular antigen in an individual by administering to the individual, preferably in nonimmunogenic form, at least one therapeutic composition of the present invention in an amount effective to render at least a portion of, and preferably all of the T cells of the individual which are specific for said

antigen unresponsive to said antigen. At least two different therapeutic compositions of the invention can be administered simultaneously or sequentially to the individual. By rendering at least a portion of the T cells unresponsive to the antigen, at least a portion of the antigen specific antibody response is inhibited. T cell epitopes are believed to be involved in initiation and perpetuation of the immune response to a protein or protein complex antigen which is in turn responsible for the clinical symptoms of disease. These T cell epitopes are thought to trigger early events at the level of the T helper cell by binding to an appropriate HLA molecule on the surface of an APC and stimulating the relevant T cell subpopulation. If the relevant T cell subpopulation has been rendered unresponsive to the antigen, then various events including the activation of the B cell cascade leading to production of antibodies will not occur. If the individual is experiencing clinical symptoms indicative of sensitivity to a disease such as an allergic reaction or allergic asthma, rheumatoid arthritis, or symptoms of graft rejection, those symptoms can be arrested or diminished upon administration of the therapeutic composition of the invention.

In yet another embodiment of the invention, there is provided a method of designing a tolerizing peptide of the invention. In one method, a tolerizing peptide may be designed by first isolating an immunogenic peptide from an antigen known to be responsible for allergy, transplantation rejection or autoimmune disease. This can be done for example by using a synthetic or recombinant peptide derived from an antigen which is previously known to be an immunogenic peptide (i.e. contains at least one T cell epitope of the antigen). This can also be done by examining the structure of an antigen of interest and producing peptides (via an expression system, synthetically or

otherwise) to be examined for their ability to influence T cell responses in a population of T cells known to be sensitive to the antigen of interest, and selecting appropriate peptides which contain at least one epitope recognized by the cells. In referring to an epitope, the epitope will be the basic element or smallest unit of recognition by a receptor particularly immunoglobulins, histocompatibility antigens and T cell receptors where the epitope comprises amino acids essential to receptor recognition.

Upon isolating or determining at least one amino acid sequence which contains at least one epitope of the antigen of interest (an immunogenic peptide), substituted peptides are generated (synthetically or otherwise) by replacing each amino acid of the immunogenic peptide (also referred to herein as the wild type peptide or native peptide) with a different amino acid (which may be a conservative amino acid, an amino acid not found in nature or alanine). Therefore, each substituted peptide contains one or more substituted amino acid which is different from the amino acid at the same site of the immunogenic peptide (or wild type peptide) of interest. Examples of conservative amino acid substitutions include but are not limited to those shown in Table I.

Table I

Examples of Conservative Amino Acid Substitutes

Amino Acid	Substitutions		
Ala	Ser		
Arg	Lys		
Asn	Gln		
Asp	Glu		
Cys	Ala	Met	
Gln	Asn		
Glu	Asp		
Gly	Ala		
His	Gln	Asn	Lys
Ile	Leu		
Leu	Ile		
Lys	Arg		
Met	Leu	Cys	
Phe	Tyr		
Pro	Ala		
Ser	Thr	Ala	
Thr	Ser		
Trp	Phe	Leu	Ile
Tyr	Phe		
Val	Ile		

The subsituted peptides are then tested to determine whether they are capable of tolerizing a portion of the T cells specific for an antigen of interest. A substituted peptide which is determined to be a tolerizing substituted peptide suitable for the invention has the ability to combine with MHC on antigenic presenting cells and cause binding of T cell receptors on at least a portion of the T cells of an individual to said peptide-MHC protein complex and has at least one and preferably all of the following properties: 1) the ability to cause no detectable or minimal proliferation of at least a portion of the T cells of an individual which are specific for said antigen in an in vitro

assay utilizing said peptide and histocompatible antigen-presenting cells; 2) the ability to cause no detectable or minimal production of IL-2 in an in vitro assay utilizing a portion of the T cells of an individual which are specific for said antigen, said peptide, and histocompatible antigen-presenting cells; 3) the ability to cause no detectable or minimal production of IL-3 in an in vitro assay utilizing a portion of the T cells of an individual which are specific for said antigen, said peptide, and histocompatible antigen presenting cells; and 4) the ability to cause no detectable or minimal production of gamma IFN in an in vitro assay utilizing a portion of the T cells of an individual which are specific for said antigen, said peptide, and histocompatible antigen presenting cells.

The invention may also include any compound that mimics a tolerizing substituted peptide of the invention as described above such as a compound not composed entirely of subunits joined by peptide bonds, but joined by other linkages (e.g. thiolester bonds), providing that the non-peptide compound mimics a tolerizing peptide capable of tolerizing T cells through the binding of T cell receptors to the complex of non-peptide and MHC protein in the absence of proliferation and/or cytokine production.

Most preferably, a tolerizing substituted peptide (or compound which mimics said peptide) of the invention causes no detectable proliferation or minimal proliferation of at least a portion of a population of T cells specific for the antigen of interest and no detectable or minimal proliferation of lymphokines such as IL-2, IL-3 etc., however, a preferred tolerizing peptide of the invention is capable of stimulating upregulation of IL-2 receptor (IL-2R) and/or LFA-1 adhesion molecule production thus indicating that the substituted peptide is binding and engaging the T cell receptor in conjunction

with the appropriate class II MHC complex and causing partial activation of the T cell.

One hypothesis for why a tolerizing substituted peptide of the invention functions as described herein may be that the contact of a peptide-MHC complex with the T cell receptor triggers multiple intracellular pathways to be activated. It is possible, therefore, that the substituted peptide of the invention which must bind the T cell receptor differently than the native or wild type immunogenic peptide, stimulates fewer of these pathways than the native peptide, and the intracellular pathway triggered is that which causes the T cells to become tolerized.

The above-described tolerizing peptide may be used in a method for tolerizing at least a portion of a population of T cells in an individual which are specific for an antigen by first generating a tolerizing peptide by the methods described above and administering said peptide in a therapeutic composition in an amount effective to cause tolerization of at least a portion of said population of T cells.

The invention is further illustrated in the following non-limiting examples.

Example 1

The Th1 clone PL-17 (also designated herein as PL-17 cells) is specific for the known immunogenic peptide of the murine hemoglobin β^d minor chain, Hbβ(64-76) (also designated herein as native peptide, native immunogenic peptide or wild type peptide (Wt Hb)) in the context of I-E^k. Substituted Hb(64-76) peptides were generated by introducing conservative,

single amino acid substitutions at any one of amino acid residues 67-76 of the native peptide as is shown in Table II.

Table II

Peptide Sequence

Peptide Designation	64	65	66	67	68	69	70	71	72	73	74	75	76
Hb(64-76)	G	K	K	V	I	T	A	F	N	E	G	L	K
Ala67-Hb(64-76)				A									
Leu68-Hb(64-76)					L								
Ser69-Hb(64-76)						S							
Ser70-Hb(64-76)							S						
Tyr71-Hb(64-76)								Y					
Gln72-Hb(64-76)									Q				
Asp73-Hb(64-76)										D			
Ala74-Hb(64-76)											A		
Ile75-Hb(64-76)												I	
Arg76-Hb(64-76)													R

The Hb(64-76) peptide and substituted peptides were synthesized using a DuPont RaMPS apparatus, purified by reverse phase HPLC on a Beckman 6300 amino acid analyzer.

Using an I-E^k-transfected L cell fibroblast, DCEK-Hi7, as APC, it was found that the peptide generated by substituting Ser for Ala at position 70 (also referred to herein as S70, Ser70 or Ser70-Hb(64-76)) ablated the proliferative response completely, even at a 10-fold greater concentration than that which gives maximal proliferation (Example 2) with the native immunogenic peptide (Figs. 1a and 1b). In addition, substituted peptide Gln72 (also referred to herein as G72, Gly72-Hb(64-76)) could not stimulate PL-17 to produce cytokines. Figs. 2a and 2b show that, while the T cells

make significant amount of IL-2 when presented with native Hb(64-76), no detectable IL-2 was seen on stimulation with either of the substituted peptides. Reports by others have suggested that the production of IL-3 and IFN-γ by Th1 clones can occur by TCR engagement alone while an additional costimulus is required for IL-2 production . Therefore, supernatants were analyzed (Example 2) after stimulation with the peptide analogs for IFN-γ (Figs. 3a and 3b) or IL-3 (Fig. 4) production (Example 2). However, as for the IL-2 profile, only the native immunogenic peptide Hb(64-76) could provide appropriate activation to allow PL-17 to synthesize IFN-γ (Figs. 3a and 3b) and IL-3 (Fig. 4). When fresh B10BR/SgSnj spleen cells were used as APC in analogous experiments similar results were found (data not shown).

One of the earliest intracellular events to occur after TCR engagement is activation of phospholipase C which catalyses the breakdown of membrane phospholipids and generation of inositol phosphates. As discussed in the above hypothesis, it is possible that an earlier activation event than cytokine production might be stimulated by the substituted Hb peptides. Thus, after stimulating the T cells with native Hb(64-76) or one of the substituted peptides (Ser70 or Gly72) and DCEK-Hi7 cells PL-17 cells were assayed for total inositol phosphate production (Example 2). Although the native Hb964-76) peptide allowed production of significant levels of inositol phosphates, the substituted peptides Ser70 and Gln72 did not (Fig. 6). Thus, for the Th1 clone PL-17, introducing conservative amino acid substitutions at various residues of the native immunogenic peptide, resulted in loss of the activation signals that stimulate detectable proliferation, cytokine production, and inositol phosphate generation.

Further, it is known that upon activation. T cells upregulate various cell surface molecules. including LFA-1 and IL-2R. Therefore a comparison of the levels of these molecules on PL-17 after stimulation with Hb(64-76). Gln72 or Ser 70. FACScan (FACS) analysis (Example 3) revealed that, while stimulation with Gln72 generally did not increase either receptor above control levels, Ser70 stimulation upregulated both LFA-1 (Fig. 10) and IL-2R (Fig. 9) levels significantly. These results suggested that Ser70 was delivering a partial signal to PL-17 upon TCR engagement that was independent of inositol phosphate generation. These results further suggest that Gln72 was not binding to the T cell receptor which is a requisite of a tolerizing substituted peptide of the invention as described above.

With the hypothesis that partial T cell stimulation might lead to tolerance induction instead of T cell activation and proliferation, the ability of the T cells to respond to the immunogenic peptide after being presented with the substituted peptides Ser70 and Gln72 was shown. A challenge proliferation assay was conducted with PL-17 cells that had been previously stimulated with DCEK-Hi7 (Fig. 11a) or B10.BR/SgSnj spleen cells (Fig. 11b) as a source of live, functional APC alone, or with Ser70 or Gln72 peptide analogs. Although T cells which had previously seen Gln72 analog responded normally in the challenge assay, PL-17 which had been previously presented with Ser70 analog were now completely unresponsive to the immunogenic peptide (Fig. 11a and Fig. 11b), although they could respond well to exogenous IL-2 (data not shown) or PMA and Ionomycin (data not shown). These results suggest that a substituted peptide which differs from the native immunogenic peptide only by a single conservative amino acid substitution is capable of transducing a partial signal to the T cell (partial activation of the T

cell) that causes it to become unresponsive to subsequent challenge with the native immunogenic peptide and is therefore deemed unresponsive and tolerized. The observation was extended further by studying the tolerizing ability of another substituted peptide, Asp73, which had given similar results as Ser70 in proliferation, cytokine production and FACS assays (data not shown). PL-17 cells which had previously been presented Asp73 were completely unable to proliferate when challenged with Hb(64-76) peptide. However, it appeared that more of Asp73 substituted peptide than Ser70 substituted peptide was needed to induce this anergic state (at least 30μM Asp73 compared to 10μM Ser70) suggesting that perhaps changes to these two positions of the native peptide have a different effect on the manner in which the TCR is engaged by the substituted peptide-MHC complex.

Next, studies investigating how long-term this state of unresponsiveness could last were conducted. When T cells were rested for 1, 3, 5, and 7 days (Example 5) before challenging with Hb(64-76) they still could not mount a proliferative response (Figs. 5a-5d), leading to the conclusion that the substituted peptide Ser70 had induced PL-17 into a state of profound anergy. In similar experiments (Example 6), it was shown that varying concentrations including relatively low concentrations of Ser70 were able to induce tolerance (Figs. 7a and 7b). However, the results in Fig 7a indicate that Gln72 was unable to induce tolerance indicating that it is not binding to the TCR and partially activating the T cells as is required of a tolerizing substituted peptide of the invention.

In previous studies, using chemically fixed APC (i.e. APC treated with ECDI) and immunogenic peptide to induce anergy, it was shown that addition of cyclosporin A or allogenic spleen to the culture prevented the T cells from

becoming unresponsive to subsequent challenge with live fixed APC . Similarly in the present system it was found that addition of cyclosporin A with Ser70 prevented this induction of anergy (Fig. 8), although adding T-depleted allogenic spleen cells as a source of costimulation had no effect (data not shown). These results suggested that the mechanism of anergy induced by substituted peptide Ser70 on a professional APC is similar to, but also distinctly different from, that caused by presentation of peptide by a fixed APC.

In other studies it was shown that non-immunogenic peptide analogs could function in an antagonistic manner by competing for TCR sites without transducing any detectable signal to the T cell. However, in this system Ser70 causes the T cells to become anergized and therefore must be sending some signal to the T cell whereas Gln 72 is not anergizing T cells and is therefore not sending a signal to the T cell. The underlying mechanism of how this is occurring is as yet unknown. However, since costimulation from APC is provided in this system, this tolerization occurs even in the presence of costimulatory signals.

One might envisage that contact of a peptide - MHC complex with a TCR triggers multiple intracellular pathways to be activated, each perhaps connected to different chains of the CD3 complex. In support of this idea, many recent studies have shown that several of the molecules that make up the CD3 complex contain functional domains and are capable of signaling independent of the rest of the complex . It is possible, therefore, that the substituted peptides, which must bind the TCR differently than the immunogenic peptide, stimulate fewer of these pathways than the native peptide and, for Ser70 and Asp73, the intracellular pathway triggered is that

which causes anergy. If this is true, then the signal for anergy induction is a normal part of the T cell response to all antigenic stimuli but is normally over-ridden by the stimulatory pathways which allow a proliferative response. By selecting substituted peptides that are recognized only slightly differently by the T cell than the native peptide one is able to separate these activation pathways.

Example 2

Studies showing PL-17 Th 1 clone (PL-17 cells) proliferative, lymphokine and inositol generation responses to substituted peptides of Hb (64-76) of the invention were conducted.

The proliferation assay (results shown in Figs 1a and 1b) was performed in 96 well flat bottomed plates in 200μl RPMI-1640 media containing 10% fetal calf serum (Hyclone, Logan, UT), 2 mM glutamine, 50μg/ml gentamicin, 10mM Hepes buffer, and 2-ME (2×10^{-5}M). PL-17 clone at 2.10^4 cells/well, mitomycin-C treated DCEK-Hi7 L cell fibroblasts (77μg/ml in HBSS for 90 minutes at 37°C, Sigma Chemical Co., St. Louis, MO) transfected with the I-E^k construct at 5×10^4 cells/well, and Hb peptides (0-100μM) were added to the appropriate wells. The assay was incubated at 37°C for 72 hours with the addition of ^{3}H-thymidine (0.4μlCi/well) during the last 20 hours. The results shown in Figs 1a and 1b indicate that the substituted peptide Ser70 ablated the proliferative response of the T cells even at a 10 fold greater concentration than that which gives maximal proliferation with the immunogenic peptide Hb(64-76). In addition, substituted peptide Gln72 could not stimulate proliferation.

IL-2 (Figs. 2a and 2b) and gamma IFN (Figs 3a and 3b) lymphokine responses were assessed using 24-hour (IL-2) or 48-hour (gamma IFN) supernatants from the above cultures. For IL-2 detection, the CH-27 B cell lymphoma (mitomycin-C treated as above, 3-5x10^4 cells/well) was used as APC instead of L cell fibroblasts to increase assay sensitivity. IL-2 was quantitated in a bioassay as proliferation of the IL-2 dependent cell line CTLL. Briefly, CTLL cells (5x10^3 cells/well) were incubated with test supernatants for 48 hours. Tritated-thymidine was included during the final 20 hours. The results in Figs 2a and 2b indicate that while the cells make a significant amount of IL-2 when presented with Hb(64-72), no detectable IL-2 was seen on stimulation with either of the substituted peptides.

Gamma IFN was measured using an ELISA as described with reagents provided by R. Schreiber (Washington University, St. Louis, MO). Monoclonal antibody specific for gamma IFN (H22) was adsorbed onto Immulon 2 (Dynatech) 96-well plates overnight at 4°C in carbonate buffer pH9.6. Bound gamma IFN was identified with a polyvalent rabbit anti-murine gamma IFN followed by peroxidase conjugated goat anti-rabbit IgG (TAGO). The substrate was developed with the ABTS reagent and read at 414 nm. As shown in Figs 3a and 3b, the results indicate that only the immunogenic peptide Hb(64-76) could provide the appropriate activation to allow PL-17 cells to synthesize gamma IFN.

IL-3 was quantitated in a bioassay as proliferation of the IL-3 dependent cell line GG1.12 (J. McKearn, Monsanto, Chesterfield, MO) ( ). Briefly, GG1.12 cells (1x10^4 cells/well) were incubated with test supernatants discussed above (collected at 48 hours) for 48 hours, with ^{3}H-thymidine included during the last 20 hours. As shown in Fig. 4, the results indicate that

only the immunogenic peptide Hb(64-76) could provide appropriate activation to allow PL-17 to synthesize gamma IFN.

For inositol phosphate generation detection PL-17 cells were incubated overnight at 1-2x10^7 cells/ml in Inositol-free RPMI containing 20-50µCi/ml myo [2-^{3}H] inositol (Amersham) and 10% FCS (dialised in PBS to remove inositol). The cells were then washed in HBSS and resuspended in RPMI 1640 complete media and 10mM LiCl (an inositol-1-phosphatase inhibitor). The T cells at 7-10x10^5 cells were incubated in 96 well flat bottomed plates with the indicated doses of Hb(64-76) or peptide analogs and DCEK-Hi7 cL cells (5.10^4/well) or irradiated B10.BR/SgSnj spleen cells(5.10^5/well) for 90-120 minutes. The samples were assayed for accumulation of free inositol phosphates. Briefly, the cultures were extracted with 1ml of a 1/2 mixture of chloroform and methonol followed by 0.25ml of chloroform and $H_2$0. The phases were separated by centrifugation and the H_2O-soluble fraction placed on a 0.25ml AG1-X8 formate ion-exchange column (Bio-Rad), then washed extensively with 5mM myo-inositol. Total free inositol phosphate was eluted from the column with 0.1M formic acid, 1M sodium formate in a volume of 1.5ml, and the ratio label was quantitated by scintiliation counting. As shown in Fig 6, the results indicate that although the immunogenic peptide Hb(64-76) allowed production of significant levels of inositol phosphates, the substituted peptides Ser70 and Gln 72 did not.

For some proliferation and lymphokine assays the L cell transfectants were substituted by irradiated B10.BR/SgSnj spleen cells (2000 rads, 5x10^5 cells/well).

Example 3

Studies using a FACscan analyzer were used to determine the presence of LFA 1 adhesion molecule and IL-2 receptors (IL-2R) on PL-17 cells and the results are shown in Figs 9 and 10. PL-17 ($5x10^5$ cells/well) were incubated with mitomycin C treated DCEK/Hi7 L cells ($5x10^5$ cells/well) alone or with the analog peptides Ser70-Hb(64-76) or Gln72-Hb(64-76) at 50μM in 24 well plates for 48 hours. The T cells were then separated from the L cells by centrifugation over Ficoll-Paque (1.077g/ml, Pharmacia LKB, NJ) at 300 rpm for 15 minutes, and washed three times before preparing for FACS analysis. For each group 1.10^5 cells were incubated for 30 minutes on ice with a, FO441.9 (anit-LFA1, Rag IgG), or b, anti-IL-2R (rat IgG, E., Unanue, Washington University, St. Louis, MO), washed twice in PBS containing o.5% BSA and 0.1% sodium azide, incubated for 30 minutes on ice with FITC-labelled goat anti-rat IgG (Southern Biotech.), then washed twice again and resuspended in PBS as above. The cells were analyzed on a FACScan analyzer.

As shown in Figs 9 and 10, the results indicate that Hb (64-76) and Ser70 Hb (64-76) stimulation upregulated both LFA-1 (Fig 10) and IL-2R (Fig. 9) levels significantly. These results suggest that Ser70 was delivering a partial signal to PL-17 upon TCR engagement that was independent of inositol phophate generation.

Example 4

Studies in the form of tolerance assays to determine whether substituted peptide Ser70-Hb(64-76) induces anergy of PL-17 were conducted and the results are shown in Figs. 11a and 11b.

PL-17 cells ($5x10^5$/well) and mitomycin-C treated (Fig. 11a). DCEK-Hi7 L cell transfectants ($5x10^5$/well) or (Fig. 11b). B10.BR/SgSnj spleen cells ($5x10^6$/well) were incubated alone or with 50μM of the indicated analog peptide for 20-24 hours at 37°C in 24 well tissue culture plates in a final volume of 700μl. The T cells were then separated form the APC by centrifugation over Ficoll-Paque (1.077g/ml. Pharmacia LKB, NJ) at 3000 rpm. for 15 minutes, washed three times in HBSS and rested for three days in 48 well tissue culture plates in RPMI media. T cells were then challenged in a proliferation assay as described in the Example 2. using Hb(64-76) as antigen (Fig. 11a). Mitomycin-C treated DCEK-Hi7 L cell transfectants or CH-27 cells (3-$5x10^4$/well) can replace the spleen cells in the challenge assay. (data not shown)

As shown in Figs. 11a and 11b, the results indicate that although T cells which had previously seen Gln72 responded normally in the challenge assay, PL-17 which had been previously presented with Ser70 were now completely unresponsive to the immunogenic peptide Hb(64-76).

Example 5

Studies showing that Ser70 -induced anergy is a) long-term and b) prevented by Cyclosporin A were conducted and the results are shown in Figs. 5a-d and in Fig 8.

PL-17 cells were used in the tolerance assay with Mitomycin C treated DCEK-Hi7 L cells as described in Example 4 with the addition of Cyclosporin A (1μg/ml) (Pharmacia) to those cells used in the studies shown in Fig. 8. T cells were separated from APC by ficoll as described in Example 2. The T cells were then rested in 48 well tissue culture plates for a)1 day (Fig 5a) b) 3

days (Fig. 5b), c) 5 days (Fig. 5c) and d) 7 days (Fig. 5d) before being challenged in a proliferation assay as described in Example 2.

The results in Figs 5a-5d indicate that even though T cells had been rested for up to seven days before challenging with Hb(64-76), they still could not mount a proliferative response which indicates that the substituted peptide Ser 70 had acted to induced PL-17 into a state of tolerization. Furthermore, the results shown in Fig 8 indicate that the addition of cyclosporin A to the culture prevented T cells from becoming unresponsive to subsequent challenge with live APC.

Example 6

Studies showing that various concentrations of the substituted peptide Ser70 of the invention is capable of inducing tolerization were conducted and are shown in Figs 7a and 7b.

PL-17 were used in tolerance assays as described in Example 5 except that the T cells were incubated overnight with various concentrations of substituted peptides Ser 70 (S70) (Fig. 7a, and 7b) and Gln 72 (Q72) (Fig 7a only) in the presence of APC.

The results shown in Fig 7a indicate that those T cells incubated with Ser70 were tolerized whereas those T cells incubated with no antigen or with Gln72 were not tolerized. The results shown in Fig. 7b indicate that those T cells incubated with very low concentrations of Ser70 were still tolerized even at high concentrations of immunogenic peptide Hb(64-76)

CLAIMS

1. A therapeutic composition comprising a pharmaceutically acceptable carrier or diluent and at least one tolerizing peptide which when administered to an individual in a therapeutically effective amount is capable of tolerizing at least a portion of the T cells of said individual which are specific for an antigen, said peptide being characterized by causing the binding of T cell receptors to complexes of the tolerizing peptide and major histocompatibility complex proteins (MHC), and being further characterized by causing no detectable or minimal proliferation of at least a portion of the T cells of an individual which are specific for said antigen in an in vitro assay utilizing histocompatible antigen-presenting cells.

2. The therapeutic composition of claim 1 wherein said peptide is characterized by causing no detectable or minimal proliferation of at least a portion of the T cells of an individual which are specific for said antigen when said peptide is administered in vivo in immunogenic form.

3. The therapeutic composition of claim 1 wherein the antigen is selected from the group consisting of an allergen, an autoantigen , and a transplantation antigen.

4. The therapeutic composition of claim 1 wherein said composition comprises at least two different tolerizing peptides.

5. The therapeutic composition of claim 1 wherein said composition comprises a sufficient number of tolerizing peptides to render substantially all of the T cells of said individual which are specific for said antigen unresponsive to said antigen.

6. A therapeutic composition comprising a pharmaceutically acceptable carrier or diluent and at least one tolerizing peptide which when administered to an individual in a therapeutically effective amount is capable of tolerizing at least a portion of the T cells of said individual which are specific for an antigen, said peptide being characterized by causing no detectable or minimal production of IL-2 in an in vitro assay utilizing a portion of the T cells of an individual which are specific for said antigen and histocompatible antigen-presenting cells.

7. The therapeutic composition of claim 6 wherein the antigen is selected from the group consisting of an allergen, an autoantigen , and a transplantation antigen.

8. The therapeutic composition of claim 6 wherein said composition comprises at least two different tolerizing peptides.

9. The therapeutic composition of claim 6 wherein said composition comprises a sufficient number of tolerizing peptides to render substantially all of the T cells of said individual which are specific for said antigen unresponsive to said antigen.

10. A therapeutic composition comprising a pharmaceutically acceptable carrier or diluent and at least one tolerizing peptide which when administered to an individual in a therapeutically effective amount is capable of tolerizing at least a portion of the T cells of said individual which are specific for an antigen, said peptide being characterized by causing no detectable or minimal production of IL-3 in an in vitro assay utilizing a portion of the T cells of an individual which are specific for said antigen and histocompatible antigen presenting cells.

11. The therapeutic composition of claim 10 wherein the antigen is selected from the group consisting of an allergen, an autoantigen , and a transplantation antigen.

12. The therapeutic composition of claim 10 wherein said composition comprises at least two different tolerizing peptides.

13. The therapeutic composition of claim 10 wherein said composition comprises a sufficient number of tolerizing peptides to render substantially all of the T cells of said individual which are specific for said antigen unresponsive to said antigen.

14. A therapeutic composition comprising a pharmaceutically acceptable carrier or diluent and at least one tolerizing peptide which when administered to an individual in a therapeutically effective amount is capable of tolerizing at least a portion of the T cells of said individual which are specific for an antigen, said peptide being characterized by causing no detectable or minimal

production of gamma IFN in an in vitro assay utilizing a portion of the T cells of an individual which are specific for said antigen and histocompatible antigen presenting cells.

15. The therapeutic composition of claim 14 wherein the antigen is selected from the group consisting of an allergen, an autoantigen , and a transplantation antigen.

16. The therapeutic composition of claim 14 wherein said composition comprises at least two different tolerizing peptides.

17. The therapeutic composition of claim 14 wherein said composition comprises a sufficient number of tolerizing peptides to render substantially all of the T cells of said individual which are specific for said antigen unresponsive to said antigen.

18. A method of treating sensitivity to an antigen in an individual comprising administering to the individual at least one therapeutic composition of claim 1 in an amount effective to render at least a portion of the T cells of the individual which are specific for said antigen unresponsive to said antigen.

19. The method of claim 18 wherein said antigen is a disease causing antigen, said tolerizing peptide causes no detectable proliferation of at least a portion of the T cells of an individual which are specific for said antigen in an in vitro assay utilizing histocompatible antigen-presenting cells and wherein said

therapeutic composition is administered to the individual during the time when the individual is experiencing clinical symptoms of the disease.

20. The method of claim 18 wherein said antigen is a transplantation antigen, said tolerizing peptide causes no detectable proliferation of at least a portion of the T cells of an individual which are specific for said antigen in an in vitro assay utilizing histocompatible antigen-presenting cells, and wherein said therapeutic composition is administered to the individual during the time when the individual is experiencing clinical symptoms of graft rejection.

21. The method of claim 18 wherein said therapeutic composition is administered to the individual in nonimmunogenic form.

22. The method of claim 18 wherein substantially all of the T cells of said individual which are specific for said antigen are rendered unresponsive to said antigen.

23 A method of treating sensitivity to an antigen in an individual comprising administering simultaneously or sequentially to the individual at least two different compositions of claim 1 in an amount effective to render at least a portion of the T cells of the individual which are specific for said antigen unresponsive to said antigen.

24. The method of claim 23 wherein substantially all of the T cells of said individual which are specific for said antigen are rendered unresponsive to said antigen.

25. A method of treating sensitivity to an antigen in an individual comprising administering to the individual at least one therapeutic composition of claim 4 in an amount effective to render at least a portion of the T cells of the individual which are specific for said antigen unresponsive to said antigen.

26. The method of claim 25 wherein the composition comprises a sufficient number of tolerizing peptides to render substantially all of the T cells of said individual which are specific for said antigen unresponsive to said antigen.

27. A method for inhibiting at least a portion of an antigen specific antibody response by the immune system of an individual comprising administering to the individual at least one therapeutic composition of claim 1 in an amount effective to render at least a portion of the T cells of the individual which are specific for said antigen unresponsive to said antigen.

28. A method of treating sensitivity to an antigen in an individual comprising administering to the individual at least one therapeutic composition of claim 6 in an amount effective to render at least a portion of the T cells of the individual which are specific for said antigen unresponsive to said antigen.

29. A method of treating sensitivity to an antigen in an individual comprising administering to the individual at least one therapeutic composition of claim 10 in an amount effective to render at least a portion of the T cells of the individual which are specific for said antigen unresponsive to said antigen.

30. A method of treating sensitivity to an antigen in an individual comprising administering to the individual at least one therapeutic composition of claim 14 in an amount effective to render at least a portion of the T cells of the individual which are specific for said antigen unresponsive to said antigen.

31. A method for tolerizing at least a portion of a population of T cells in an individual which are specific for an antigen comprising the steps of :

isolating at least one peptide derived from said antigen, said peptide comprising at least one T cell epitope;

substituting at least one amino acid residue of said peptide with a different amino acid such that the substituted peptide causes no detectable or minimal proliferation of at least a portion of the T cells of an individual which are specific for said antigen in an in vitro assay utilizing histocompatible antigen-presenting cells, but the substituted peptide is capable of tolerizing at least a portion of the T cells of an individual which are specific for the antigen when administered to said individual in a therapeutically effective amount; and

administering to the individual whose T cells you want to tolerize a therapeutic composition comprising the substituted peptide and a pharmaceutically acceptable carrier or diluent in an amount effective to cause tolerization of at least a portion of said population of T cells.

32. The method of claim 31 wherein the amino acid substitution is a conservative amino acid substitution.

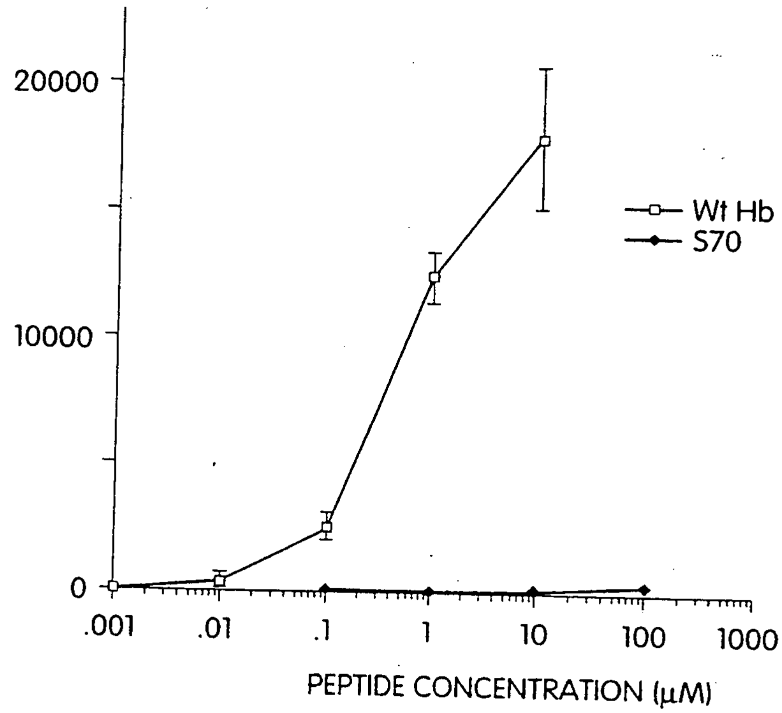
33. The method of claim 31 wherein the amino acid to be substituted is substituted with an amino acid which is not a natural amino acid found in nature.

34. A therapeutic composition of claim 1 wherein said tolerizing peptide is a compound which mimics said tolerizing peptide.

35. A method of designing a peptide which when administered to an individual in a therapeutically effective amount is capable of tolerizing at least a portion of the T cells of said individual which are specific for an antigen, said peptide being characterized by causing no detectable or minimal proliferation of at least a portion of the T cells of an individual which are specific for said antigen in an in vitro assay utilizing histocompatible antigen-presenting cells comprising the steps of:

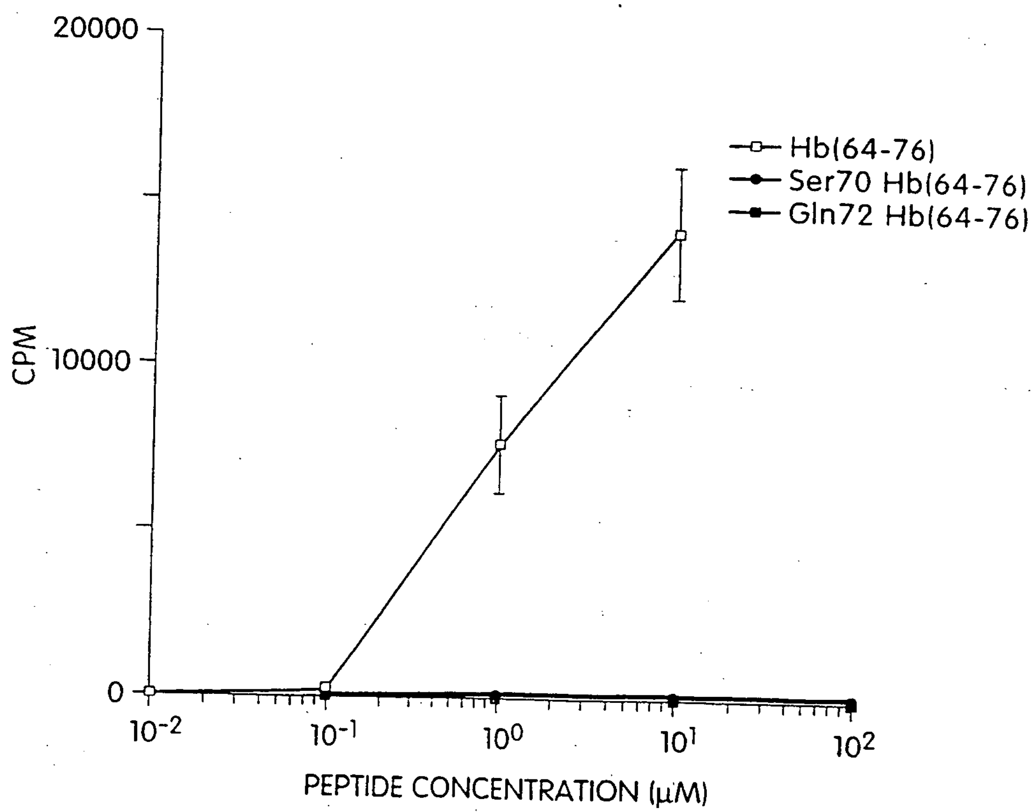
isolating at least one peptide derived from said antigen, said peptide comprising at least one T cell epitope; and

substituting at least one amino acid residue of said peptide with a different amino acid such that the substituted peptide causes no detectable or minimal proliferation of at least a portion of the T cells of an individual which are specific for said antigen in an in vitro assay utilizing histocompatible antigen-presenting cells, but the substituted peptide is capable of tolerizing at least a portion of the T cells of an individual which are specific for the antigen when administered to said individual in a therapeutically effective amount.



20000
10000
0
.001
.01
.1
1
10
100
1000
PEPTIDE CONCENTRATION (μM)
Wt Hb
S70

Fig. 1a



20000
10000
0
CPM
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
PEPTIDE CONCENTRATION (μM)
Hb(64-76)
Ser70 Hb(64-76)
Gln72 Hb(64-76)

Fig. 1b

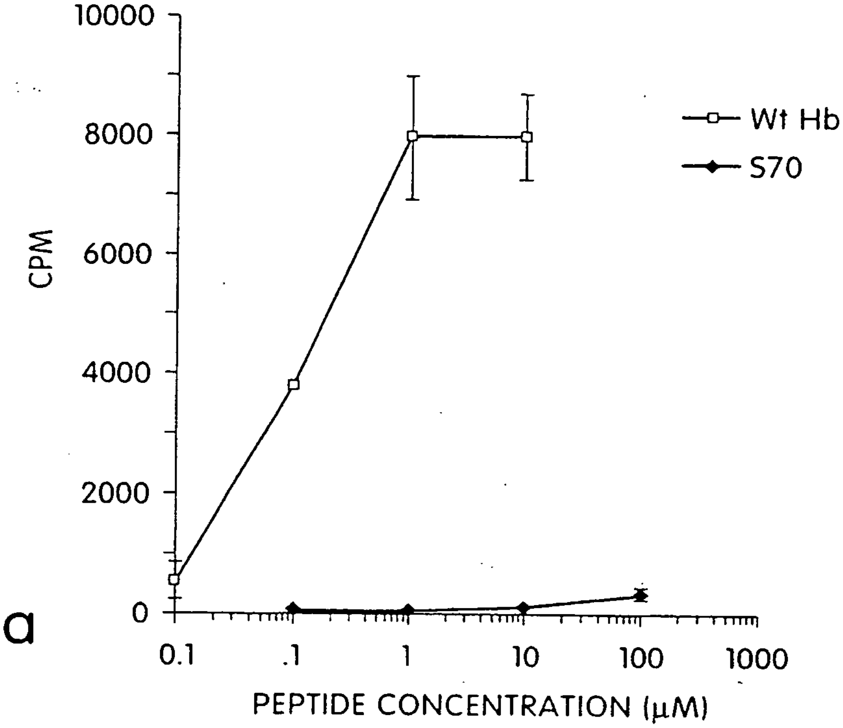

10000
8000
6000
4000
2000
0
CPM
Wt Hb
S70
0.1
.1
1
10
100
1000
PEPTIDE CONCENTRATION (μM)

Fig. 2a

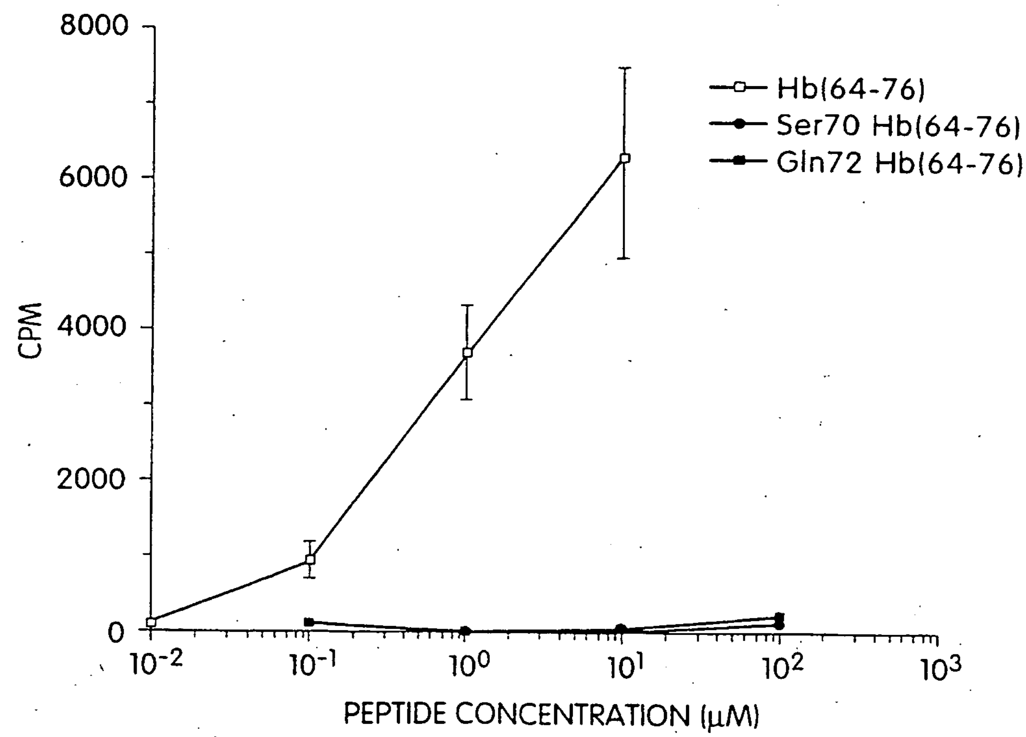

8000
6000
4000
2000
0
CPM
Hb(64-76)
Ser70 Hb(64-76)
Gln72 Hb(64-76)
10-2
10-1
100
101
102
103
PEPTIDE CONCENTRATION (μM)

Fig. 2b

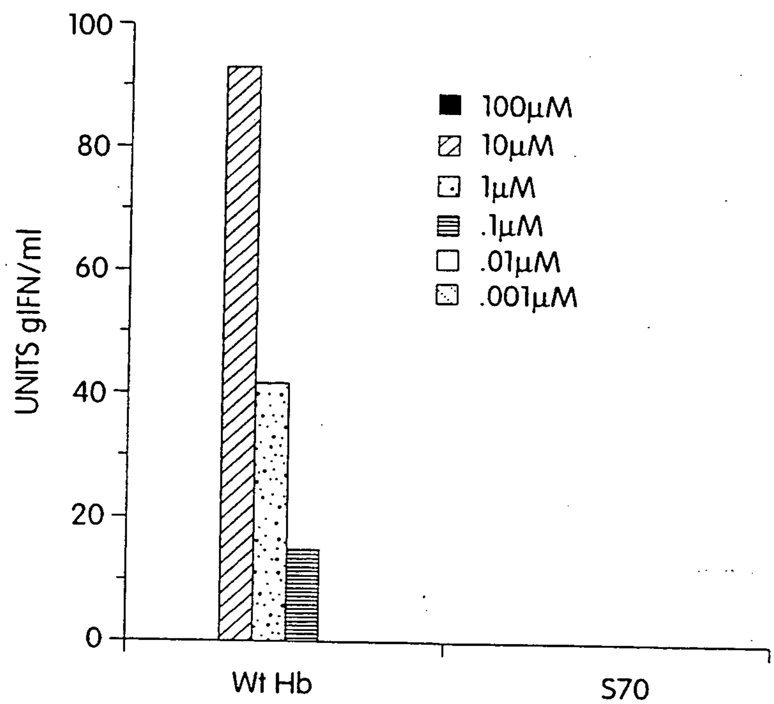

100
80
60
40
20
0
UNITS gIFN/ml
100μM
10μM
1μM
.1μM
.01μM
.001μM
Wt Hb
S70

Fig. 3a

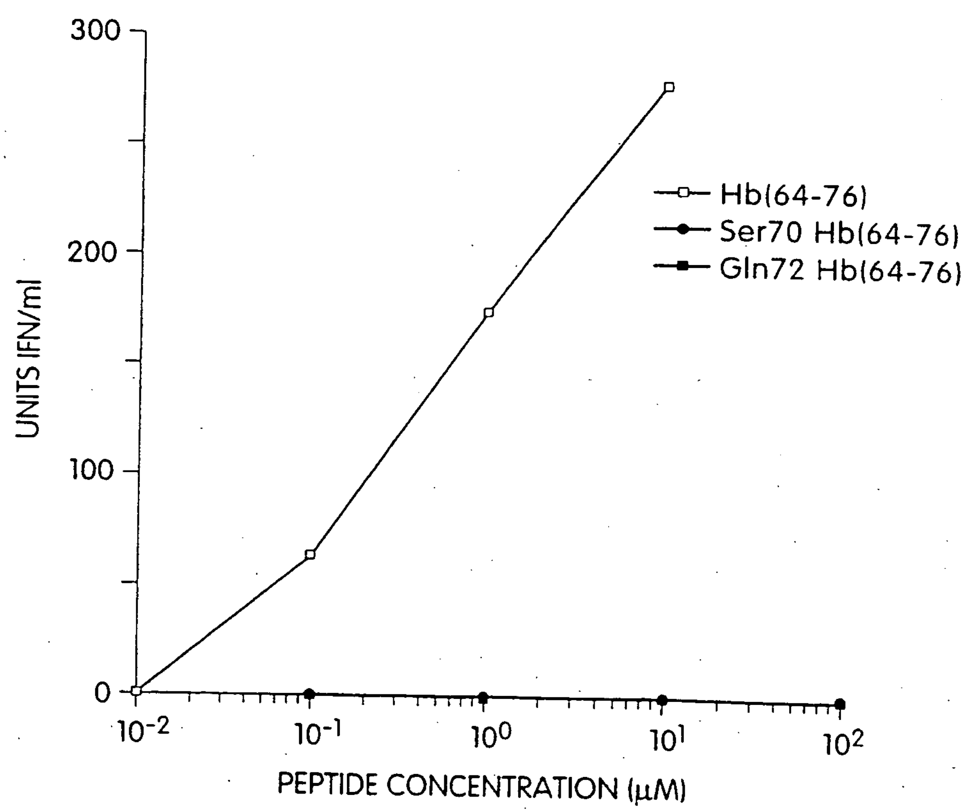

300
200
100
0
UNITS IFN/ml
Hb(64-76)
Ser70 Hb(64-76)
Gln72 Hb(64-76)
10-2
10-1
100
101
102
PEPTIDE CONCENTRATION (μM)

Fig. 3b

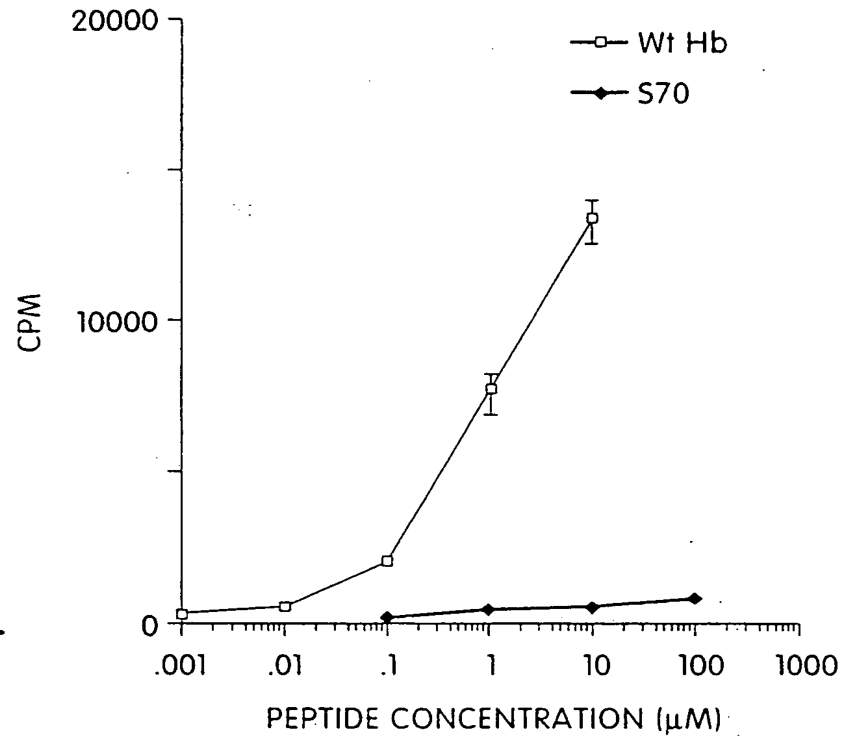

20000
10000
0
CPM
Wt Hb
S70
.001
.01
.1
1
10
100
1000
PEPTIDE CONCENTRATION (μM)

Fig. 4

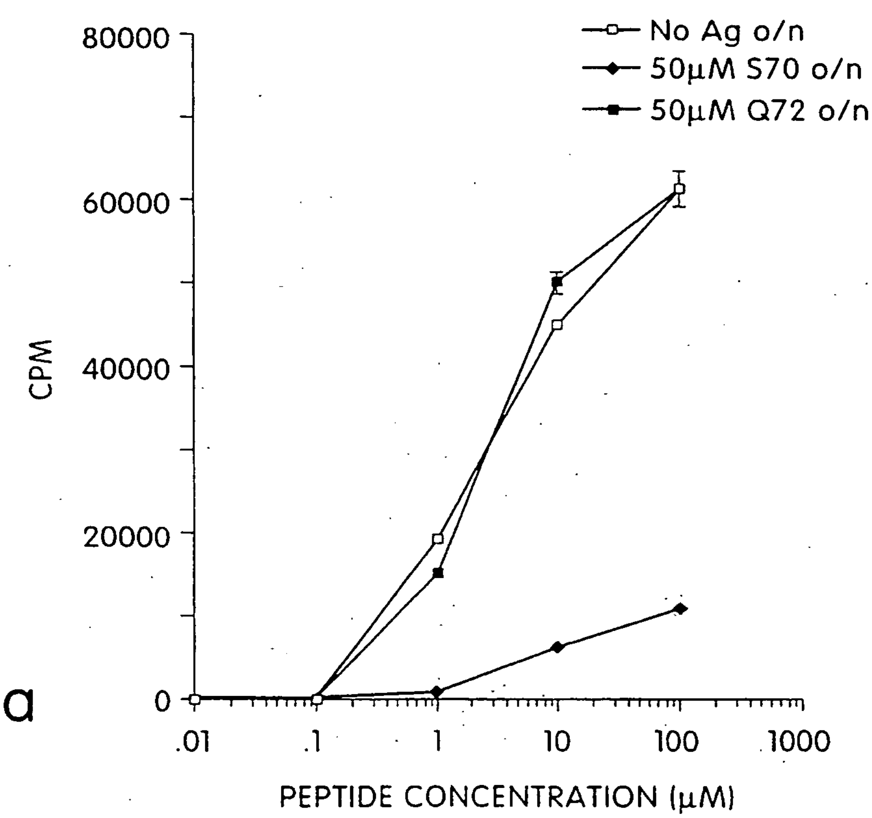

80000
60000
40000
20000
0
CPM
No Ag o/n
50μM S70 o/n
50μM Q72 o/n
.01
.1
1
10
100
1000
PEPTIDE CONCENTRATION (μM)

Fig.5a

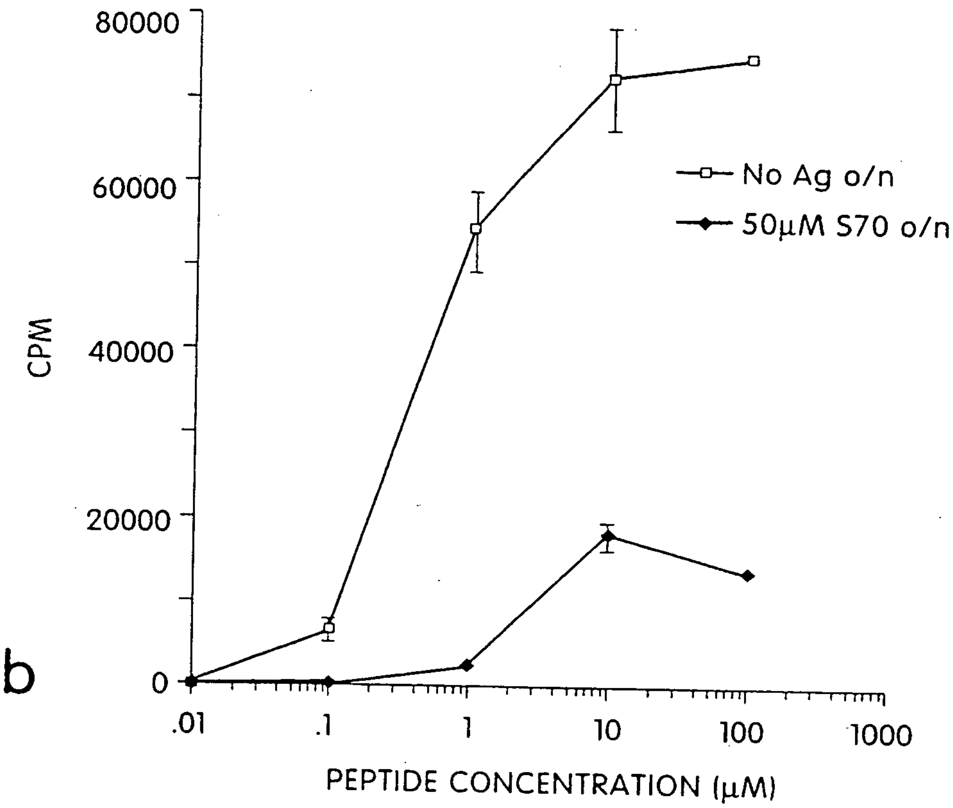


Fig.5b

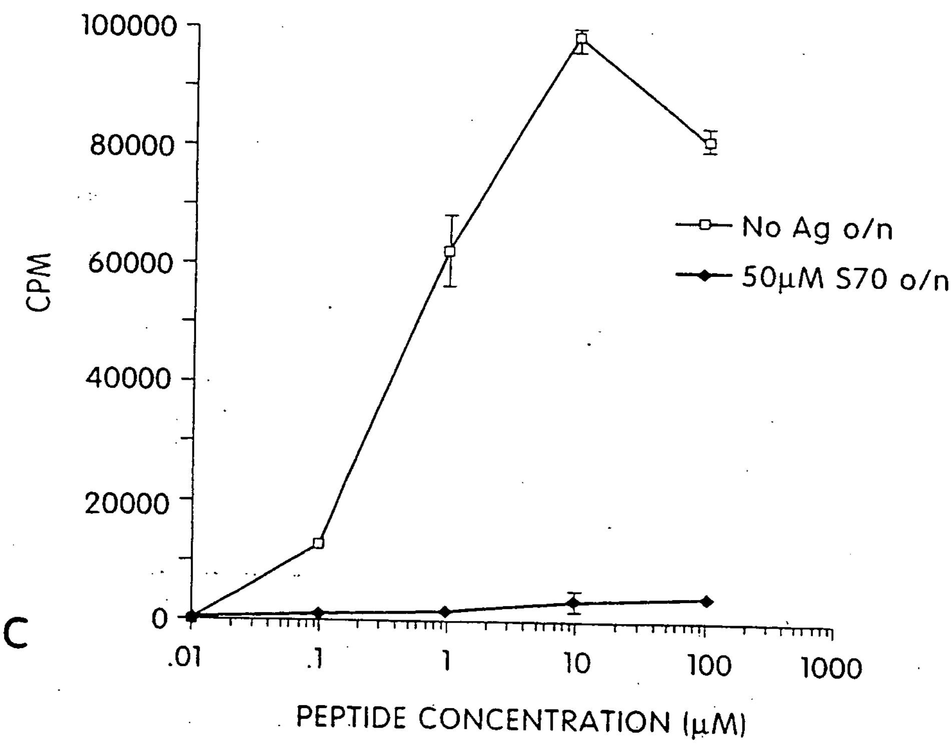


Fig.5c

SUBSTITUTE SHEET

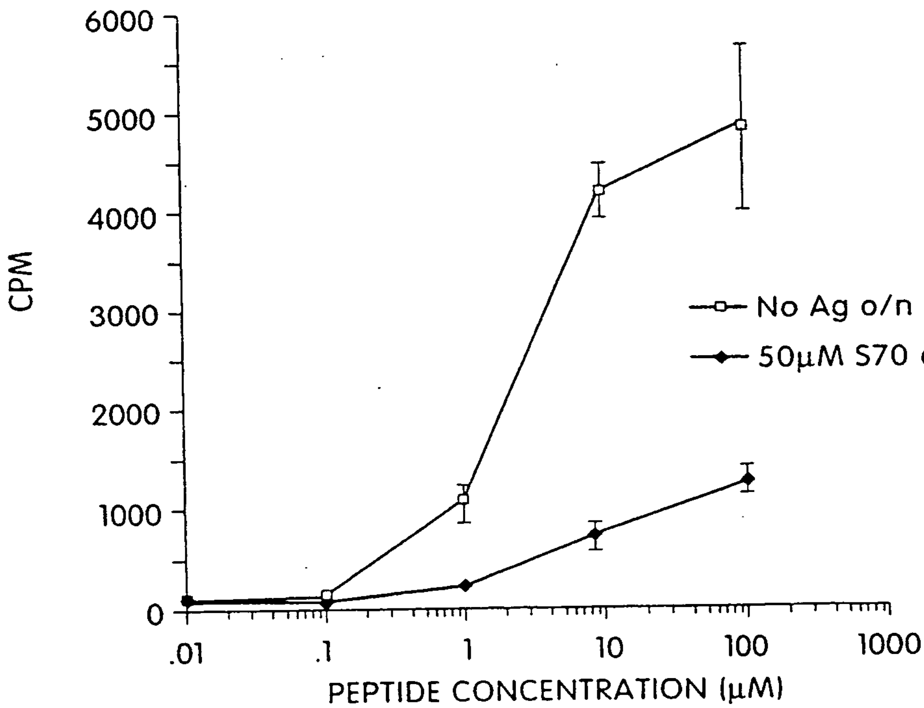

6000
5000
4000
3000
2000
1000
0
CPM
No Ag o/n
50µM S70 o/n
.01
.1
1
10
100
1000
PEPTIDE CONCENTRATION (µM)

Fig.5d

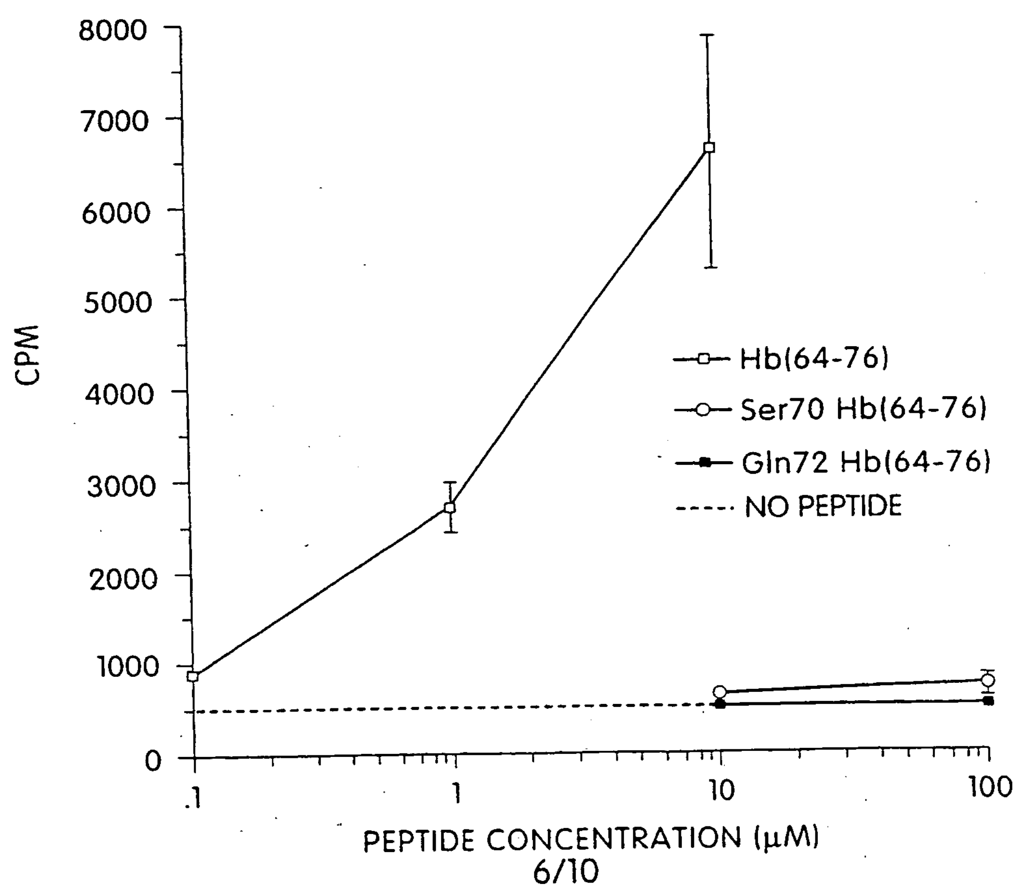

8000
7000
6000
5000
4000
3000
2000
1000
0
CPM
Hb(64-76)
Ser70 Hb(64-76)
Gln72 Hb(64-76)
NO PEPTIDE
.1
1
10
100
PEPTIDE CONCENTRATION (µM)

Fig. 6

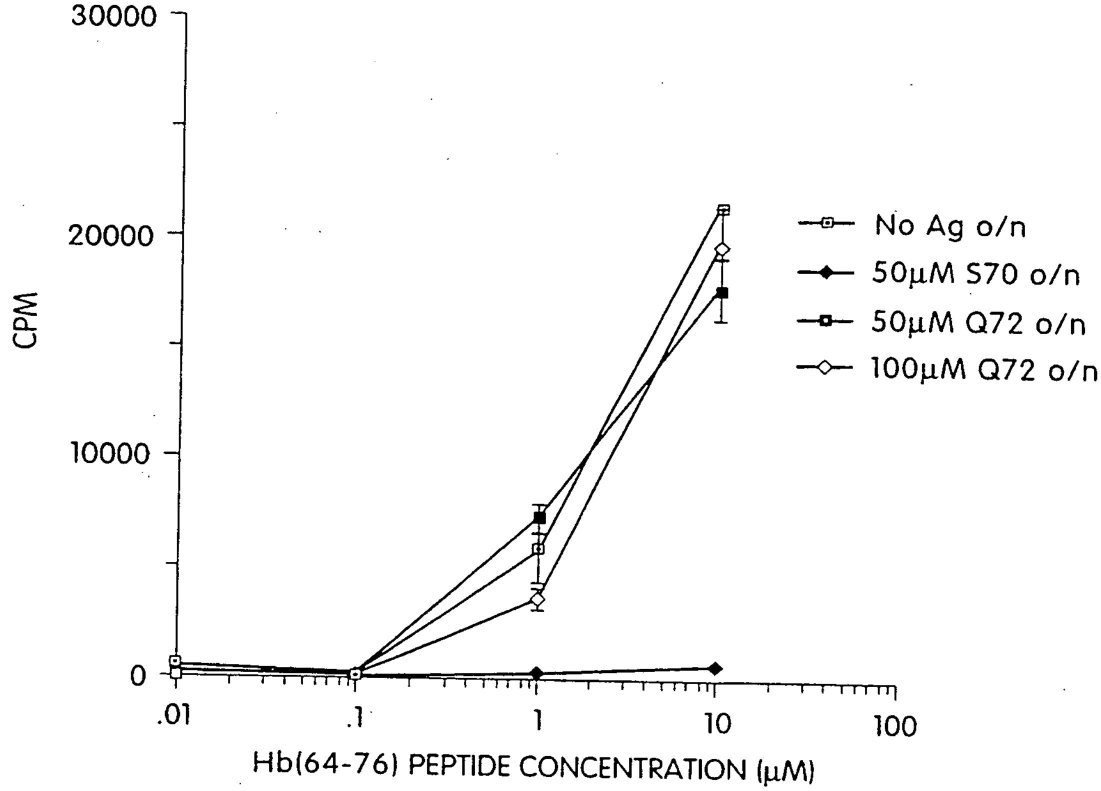
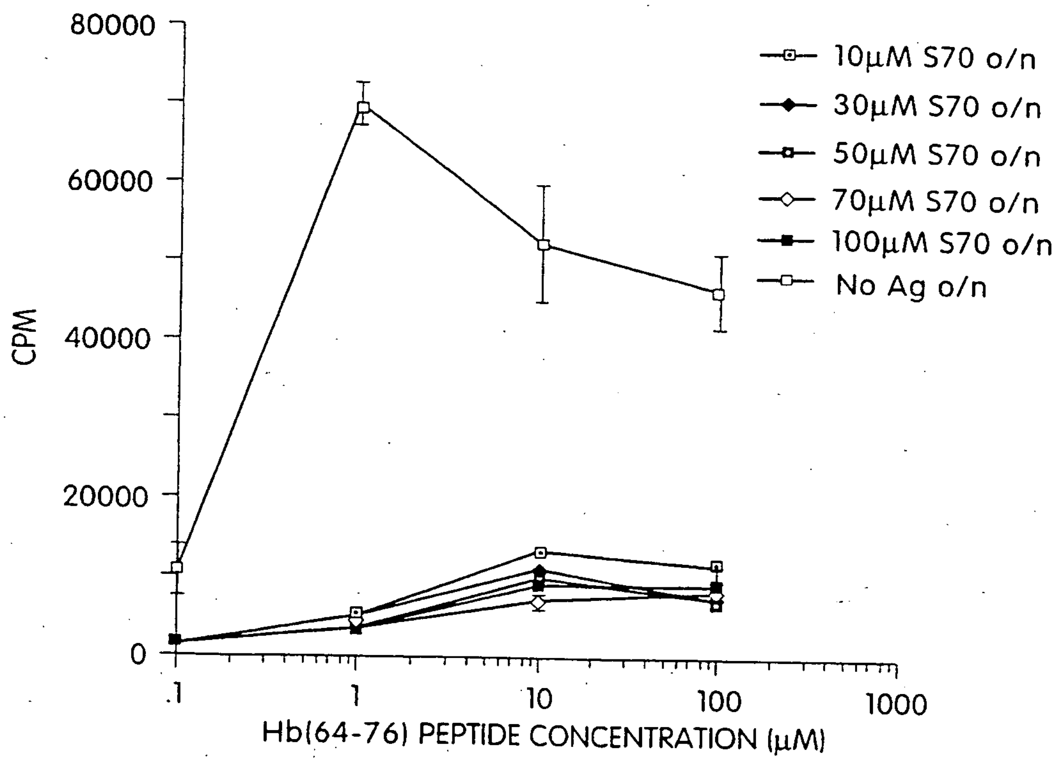


Fig. 7a



7/10 Fig. 7b

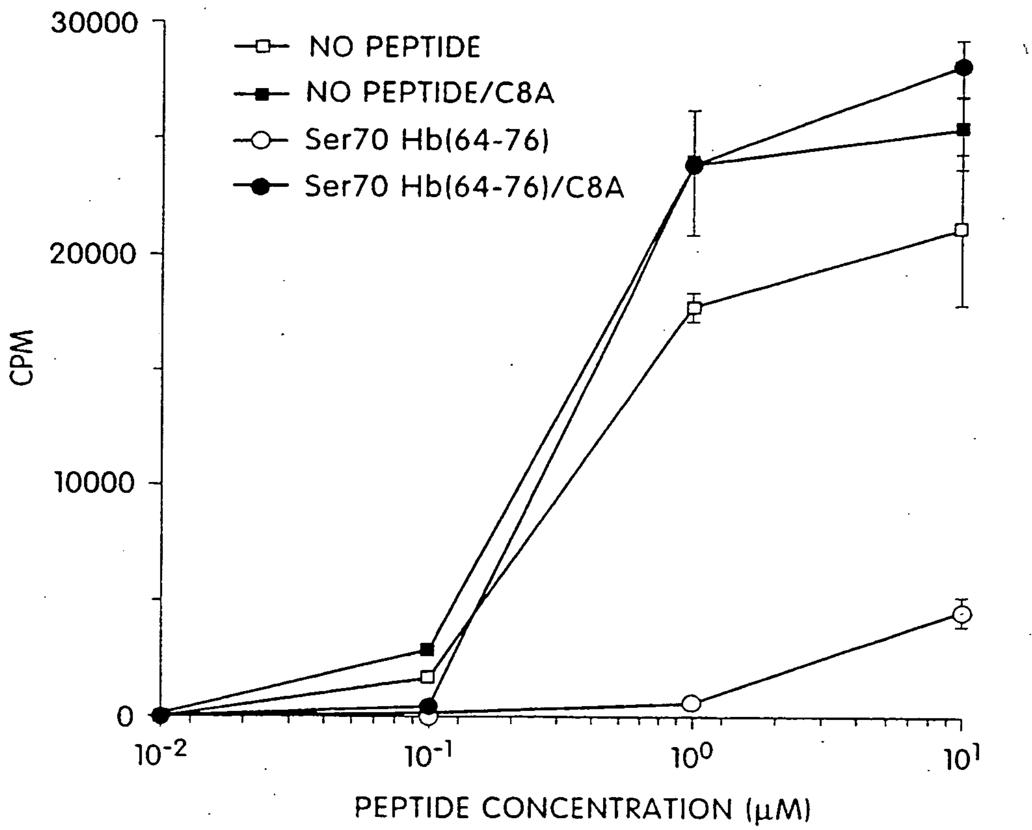

30000
20000
10000
0
CPM
10-2
10-1
100
101
PEPTIDE CONCENTRATION (μM)
NO PEPTIDE
NO PEPTIDE/C8A
Ser70 Hb(64-76)
Ser70 Hb(64-76)/C8A

Fig. 8

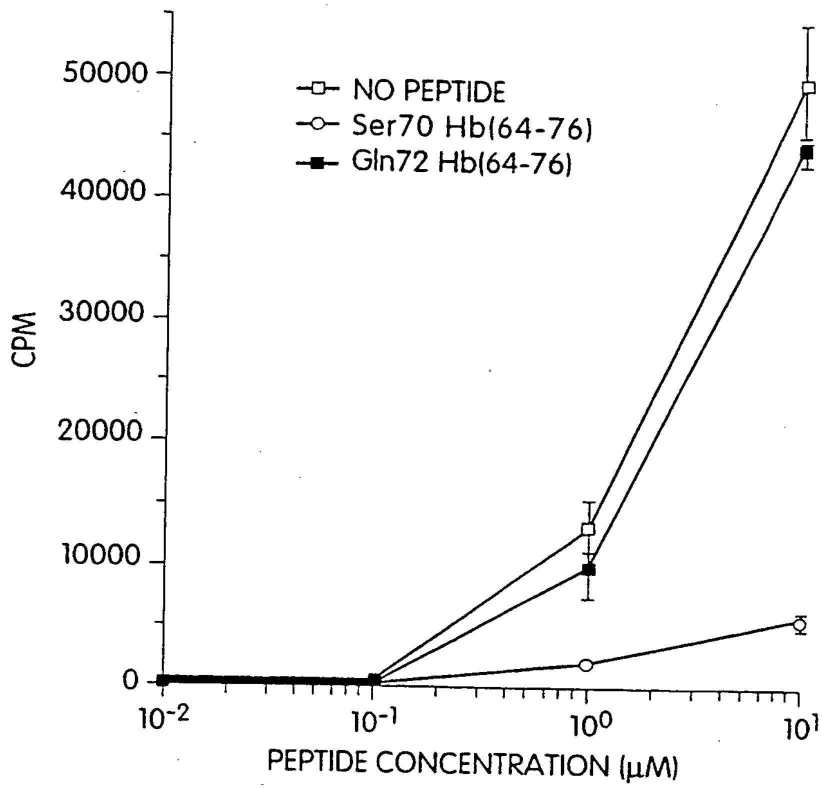


Fig. 11a

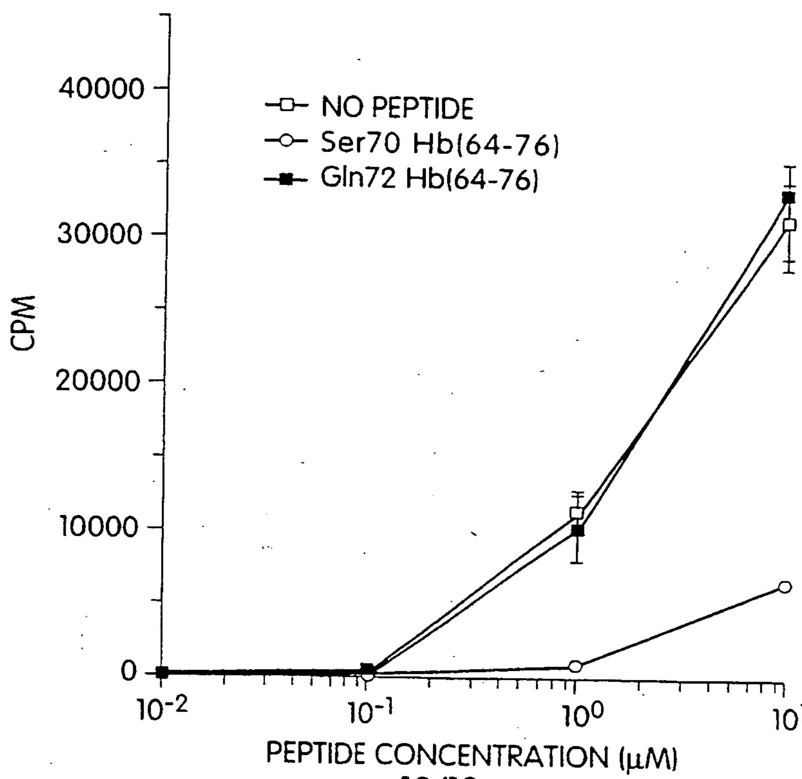


Fig. 11b

SUBSTITUTE SHEET

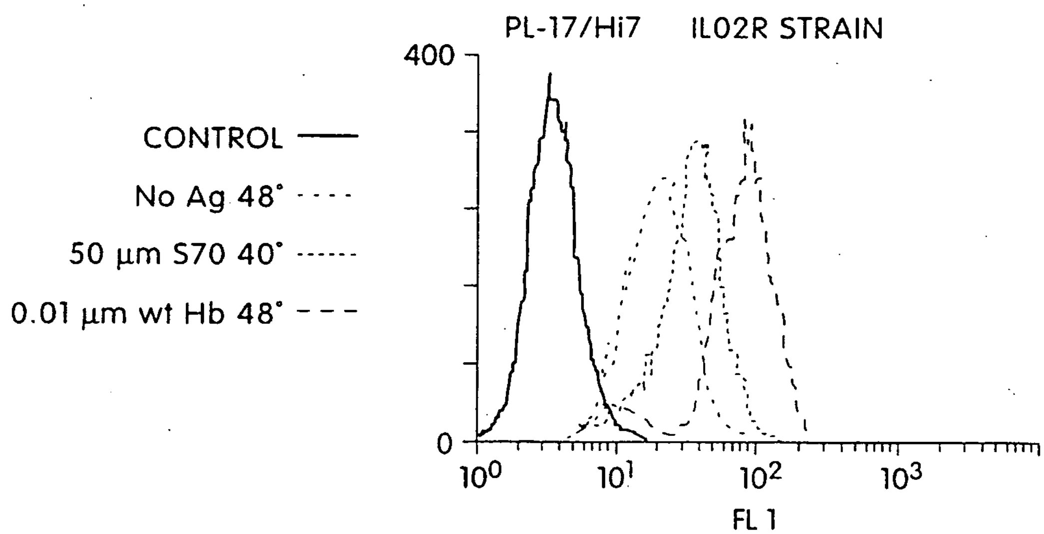

PL-17/Hi7 IL02R STRAIN
CONTROL
No Ag 48°
50 µm S70 40°
0.01 µm wt Hb 48°
400
0
10^0
10^1
10^2
10^3
FL 1

Fig. 9

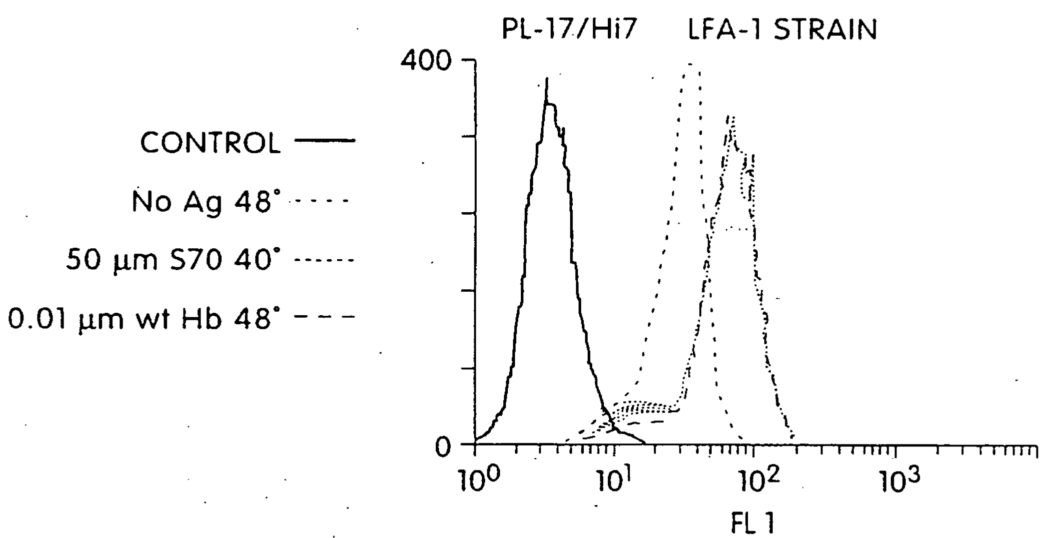

PL-17/Hi7 LFA-1 STRAIN
CONTROL
No Ag 48°
50 µm S70 40°
0.01 µm wt Hb 48°
400
0
10^0
10^1
10^2
10^3
FL 1

Fig. 10

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/08456

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K15/00; C07K7/08; A61K37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	THE JOURNAL OF IMMUNOLOGY vol. 2, 15 January 1092, USA pages 347 - 353 B.D. EVAVOLD ET AL. 'COMPLETE DISSECTION OF THE Hb(64-76) DETERMINANT USING T HELPER 1, T HELPER 2 CLONES, AND T CELL HYBRIDOMAS' see the whole document ---	1-35
X	SCIENCE vol. 252, 1991, WASHINGTON DC pages 1308 - 1310 B.D. EVAVOLD ET AL. 'SEPARATION OF IL-4 PRODUCTION FROM Th CELL PROLIFERATION BY AN ALTERED T CELL RECEPTOR LIGAND' see the whole document ---	1-35

-/--

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14 DECEMBER 1993	01 -02- 1994
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	DEFFNER C.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	SEMINARS IN IMMUNOLOGY vol. 3, 1991, USA pages 225 - 229 B.D. EVAVOLD ET AL. 'T CELL INDUCING DETERMINANTS CONTAIN A HIERARCHY OF RESIDUES CONTACTING THE T CELL RECEPTOR' *Hb 64-76* see page 227 - page 228 ---	1-35
X	PROC. NATL. ACAD. SCI. USA vol. 89, September 1992, pages 7727 - 7731 C.H. PONTZER ET AL. 'T-CELL ANTIGEN RECEPTOR BINDING SITES FOR THE MICROBIAL SUPERANTIGEN STAPHYLOCOCCAL ENTEROTOXIN A' see page 7730, right column, last paragraph; figures 4,7 -----	1-35